IMPORTANCE OF BRAIN DOPAMINE FOR THE STIMULANT ACTIONS OF AMPHETAMINE*

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IT HAS been suggested (TAYLOR and SNYDER, 1971) that the central stimulant actions of amphetamine are mediated by brain norepinephrine (NE) whereas the stereotype behaviours that follow high doses of this drug are mediated by dopamine (D). We have investigated the behavioural effects of a number of drugs that alter the dynamics of brain catecholamines and have concluded that many pharmacological effects previously attributed to modifications of NE actions result instead from alterations of dopaminergic systems. Experiments on how drugs which block catecholamine synthesis influence the actions of *d*-amphetamine will serve as one example of the studies which have led to this conclusion.

The antiamphetamine actions of α-methyltyrosine (αMT), an inhibitor of tyrosine hydroxylase (EC 1.14.3.1), are well documented (e.g. WEISSMAN *et al.*, 1966; DOMINIC and MOORE, 1969; MOORE and DOMINIC, 1971) and suggest that the central stimulant actions of *d*-amphetamine are mediated by a newly synthesised pool of brain catecholamines. Since αMT blocks the synthesis of both NE and D, it is not possible to relate the antiamphetamine properties of this drug to a disruption of synthesis exclusive to either of these two catecholamines in the brain. If the antiamphetamine effects of αMT result from inhibition of NE synthesis, then inhibition of dopamine-β-hydroxylase (EC 1.14.2.1, DBH) should duplicate the actions of αMT. On the other hand, if DBH inhibitors do not block the stimulant actions of amphetamine, then the αMT effect may result from inhibition of D synthesis.

It has been reported that DBH inhibitors produce behavioural depression and block the central stimulation produced by amphetamines and related drugs (SVENSSON and WALDEK, 1969; MAJ *et al.*, 1968). Nevertheless, the behavioural depressant and NE depleting actions of these inhibitors do not appear to be casually related (MOORE, 1969). Intraperitoneal administration of various DBH inhibitors [disulfiram; FLA-63, bis (4-methyl-l-homopiperazinyl-thiocarbonyl disulfide); U-14,624, 1-phenyl-3-(2-thiazolyl)-2-thiourea] reduces locomotor activity in rodents and markedly elevates plasma corticosterone and blood glucose concentrations (THORNBURG and MOORE, 1971). These effects may have resulted from peritoneal irritation produced by the intraperitoneal administration of the insoluble inhibitors. When administered in the diet DBH inhibitors reduced brain NE concentrations, blocked NE synthesis, but did not reduce spontaneous locomotor activity (MOORE, 1969; VON VOIGTLANDER and MOORE, 1970).

The effects of adding αMT, U-14,624, and FLA-63 to the diet of mice that have been accommodated to consuming their daily food in 4 hr are compared in Table 1.

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TABLE 1. EFFECTS OF 4 HR DIETS CONTAINING 0·4% α-METHYLTYROSINE, 0·4% U-14,624 OR 0·05% FLA-63 ON BRAIN CONTENTS OF (^{14}C) AND OF ENDOGENOUS TYROSINE AND CATECHOLAMINES AND ON LOCOMOTOR ACTIVITY IN MICE. One hr after the 4 hr test diet mice were sacrificed and their brains analyzed for endogenous compounds as described by CARR and MOORE (1968). Other mice were injected i.v. with 10 μCi L-(^{14}C) tyrosine, sacrificed 30 min later, and their brains analysed for radioactive compounds as described by BHATNAGAR and MOORE (1972). Locomotor activity was determined in the following manner. One hour after the 4 hr test diet pairs of mice were placed in Woodard actophotometers and activity recorded 10–20 min later. The mice were then injected i.p. with saline or *d*-amphetamine sulfate (2 mg/kg), returned to actophotometers and activity recorded 20–60 min later. Amphetamine-stimulated activity represents the activity of animals injected with amphetamine less the activity of animals injected with saline. Numbers represent means ± S.E.M. and underlined values are statistically different from controls ($P < 0·01$); values in parentheses represent the number of determinations.

Diet	Control	αMT	U-14,624	FLA-63
Endogenous compounds (6–18)				
Tyrosine (μg/g)	13·2 ± 1·5	14·3 ± 3·7	10·4 ± 0·4	13·1 ± 1·8
Norepinephrine (μg/g)	0·35 ± 0·01	0·22 ± 0·02	0·17 ± 0·01	0·15 ± 0·01
Dopamine (μg/g)	0·75 ± 0·04	0·31 ± 0·03	0·79 ± 0·04	0·90 ± 0·13
^{14}C-compounds (3–5)				
^{14}C-Tyrosine (dis/min × 10^2/brain)	270 ± 12	287 ± 41	300 ± 35	324 ± 22
^{14}C-Norepinephrine (dis/min/brain)	72 ± 5	24 ± 3	31 ± 4	24 ± 3
^{14}C-Dopamine (dis/min/brain)	176 ± 5	78 ± 15	372 ± 109	308 ± 22
Locomotor activity (12–42)				
Exploratory activity	647 ± 25	450 ± 31	695 ± 26	582 ± 39
Amphetamine-stimulated activity	2927 ± 229	1268 ± 78	2724 ± 439	2541 ± 384

When administered in this manner none of the drugs altered plasma corticosterone concentrations. All drugs, however, reduced the brain contents of NE, but only αMT reduced the brain contents of D. All three drugs significantly reduced the accumulation of (^{14}C)NE 30 min after the i.v. administration of L-(^{14}C) tyrosine. Only αMT reduced the brain content of (^{14}C)D, whereas the DBH inhibitors increased the brain content of this radioactive amine. None of the diets altered the endogenous or (^{14}C) tyrosine contents of the brain. Only mice on the αMT diet exhibited significantly reduced spontaneous and amphetamine-stimulated activity. Similar results have been obtained with mice placed on 24 hr diets of the inhibitors of catecholamine synthesis (THORNBURG, 1972). These results suggest, therefore, that the locomotor stimulant effects of amphetamine involve a dopaminergic mechanism.

This proposal is supported by the results of the experiment illustrated in Fig. 1. The activities of groups of 4 mice were recorded in their home cages during the 12 hr dark–light cycle. By using this procedure animal activity could be recorded with minimal disturbance to ongoing behaviour and without possible nonspecific disturbing effects that might accompany parenteral drug administration. In the top panel the first two pairs of bars represent activity on two consecutive days of control diet; this activity was set at 100 per cent. The next day the mice received a diet containing 0·4 per cent αMT and there was a slight reduction in activity. The next day 0·05 per cent amphetamine was added to the αMT diet and little effect was noted. Animals then received two days of control diet and finally a diet containing 0·05 per cent amphetamine which caused a marked increase in activity. Thus, the antiamphetamine property of αMT was clearly demonstrated.

In the bottom panel the same experimental design was utilised except that U-14,624 was added to the diet in place of αMT. U-14,624 alone had little effect on

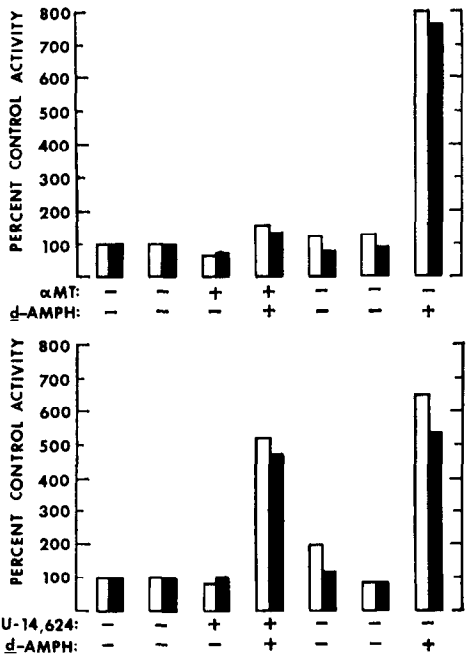

FIG. 1.—Effects of amphetamine on the activity of mice in their home cages. Activity was recorded and food was presented only during the 12 hr dark phase of a 24 hr dark–light cycle. Activity is expressed as a percentage of control activity. Open bars represent results obtained with an instrument that utilises electromagnetic proximity sensors (Selective Activity Meter, Columbus Instruments) and solid bars represent results obtained with an instrument that utilises 40 infrared-sensitive photocells (Electronic Motility Meter, Motron Products). DL-α-methyltyrosine (0·4%), U-14,624 (0·4%) and *d*-amphetamine sulfate (0·05%) were added to the diet as indicated.

activity, but when amphetamine was added to the U-14,624 diet a marked stimulation was observed that was approximately equivalent to that seen two days later when amphetamine alone was added to the diet. Thus, blockade of DBH with U-14,624 did not influence amphetamine-induced stimulation of activity. Since disruption of both NE and D synthesis by αMT blocks *d*-amphetamine-stimulated activity while disruption of only NE synthesis does not, it appears that the stimulation produced by amphetamine is primarily dependent upon a dopaminergic mechanism. This conclusion, however, is based upon the premise that antiamphetamine properties of αMT are related to the ability of this drug to inhibit tyrosine hydroxylase. It has recently been reported (ENNA *et al.*, 1973) that αMT blocks amphetamine-induced release of amines from brain slices. Using an *in situ* cerebroventricular perfusing technique, an inhibitory effect of αMT on amphetamine-induced release of (^{3}H)D from the caudate nucleus of the cat could not be demonstrated (CHIUEH and MOORE, 1973).

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